Genetic architecture of quality traits in wheat

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ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool (BLAST)
BW	Bread wheat panel
CE-DW	Central European durum wheat panel
GPD	Grain protein deviation
h^2	heritability estimate
KASP	Competitive allele-specific PCR
MAS	Marker-assisted selection
NIRS	Near infrared spectroscopy
QTL	Quantitative trait loci
RKHS	Reproducing kernel Hilbert space
RR-BLUP	Ridge regression BLUP
SDSS	Sedimentation volume determined with sodium dodecyl sulfate method
SPW	Spelt wheat panel
SWE-DW	Southwestern European durum wheat panel
TKM	Thousand kernel mass
wRR-BLUP	Weighted RR-BLUP
Z-SDS	sedimentation volume determined according to Zeleny

1 GENERAL INTRODUCTION

The genus of wheat subdivides into several species of which bread wheat (*Triticum aestivum* ssp. *aestivum*) is the predominantly grown species (FAOSTAT 2019). More than half of the bread wheat harvest is used for animal feed, and to a lesser extent for human consumption, mainly for the production of pastries. Durum wheat (*Triticum turgidum* ssp. *durum*) is another important representative of the wheat genus, although the durum world production is considerably smaller than that of bread wheat. However, in contrast to bread wheat, durum wheat is almost exclusively used for food production (Sieber et al. 2015). A large part of the global durum yield is used for the production of pasta, and to smaller amounts for bulgur and couscous. In Central Europe, the bread wheat subspecies spelt (*T. aestivum* ssp. *spelta*) is a further member of the wheat genus with a growing importance, but due to the relatively small production volume, it may still be considered as a niche market.

The wheat species differ not only in their genetic constitution, morphological appearance and in their agronomic performance; they also show in some cases considerable differences regarding their grain ingredients (Longin et al. 2016). The diverging grain composition is one major reason for the usage of the wheat species in different products. Also within species, there is a large variation, which has been used by plant breeders and has led to the release of a large number of new varieties with a large variation in their quality profiles.

MULTIPLE QUALITY DEMANDS IN DURUM WHEAT AND THE POTENTIAL OF GENOMICS-ASSISTED BREEDING

The requirement to combine several quality parameters is particularly high for durum wheat, since the majority of durum wheat food products, such as pasta, bulgur, and couscous are little processed and in most cases do not contain further ingredients that would allow adjustments. A high quality of the grain not only ensures a high quality of the final product, it is also necessary for manufacturers to obtain a good processing quality and a high product yield. Consequently, durum millers and pasta producers track and control intensively numerous quality traits within the product chain.

Among the important quality traits asked by durum millers are protein content, sedimentation volume, vitreousity, falling number, and thousand kernel mass (Samaan et al. 2006; Sieber et al. 2015). The protein content and the sedimentation volume, as measure for the protein quality are of high importance for pasta producers as they influence the firmness of cooked pasta (“al dente”; Samaan et al. 2006; Bruneel et al. 2010; Fois et al. 2011; Kaur et al. 2015). A low falling number, occurring mainly at humid harvest conditions, is an indication for a high alpha-amylase activity, which is associated with the beginning of the germination process and the degradation of starch (Dick et al. 1974; Dexter et al. 1989). Often, low falling numbers lead to brown instead of light yellow pasta due to maillard reaction products formed during pasta production and drying (Sissons 2008; Fu et al. 2014). The vitreousity, representing the glassy

appearance of durum grains, and the thousand kernel mass (TKM) influence the semolina yield and are therefore of special importance for the durum millers (Matsuo and Dexter 1980; Sieber et al. 2015). Thus, a successful durum variety should combine high values of protein content, sedimentation volume, falling number, vitreousity and TKM.

In order to provide such varieties to the market, plant breeders intensively phenotype and select on these quality traits in durum wheat breeding programs. Protein content can be evaluated fast and easily with near infrared spectroscopy (NIRS) already on grains on the combine harvester or in the lab. However, the moderate heritability of protein content requires phenotyping at multiple environments to obtain reliable data (Blanco et al. 2006, 2012). Likewise, vitreousity and falling number largely depend on the environmental conditions close to harvest with continuous humid conditions leading to lower values of both traits (Dick et al. 1974). In contrast, under dry harvest conditions there is often only little variation among durum lines for falling number and vitreousity, so that meaningful selection is difficult. Thus, the selection for falling number and vitreousity is challenging especially in regions with yearly changing and area-specific rainfalls at harvest time, as is the case in Central Europe. In addition, for the evaluation of falling number and sedimentation volume, milling of the grain is required before lab analyses. Accounting in breeding programs for the mentioned quality-related requirements is complex and thus the use of genomics-assisted breeding for these traits opens up great opportunities to facilitate and speed up durum breeding.

In the literature, few studies cover the genetic architecture of quality traits in durum wheat. For protein content, no major quantitative trait loci (QTL) could be identified but prediction abilities from genomic selection were of promising magnitude (Blanco et al. 2006; Suprayogi et al. 2009; Fiedler et al. 2017; Haile et al. 2018). For sedimentation volume, a major QTL possibly associated with the *Glu-B3* locus was detected in a bi-parental mapping population on chromosome 1B explaining a large proportion of the phenotypic variance (Kumar et al. 2013). Consistently, in a recent study in North American durum breeding germplasm QTL mainly on chromosome 1A and 1B in a similar region were identified (Fiedler et al. 2017). However, the identified QTL of this study explained only a minor proportion of the phenotypic variance and it was concluded that most major gene loci related to quality were fixed in the North American elite breeding germplasm. While to my knowledge for falling number in durum wheat no studies about the genetic architecture are available, a few studies approach the genetic architecture of the related trait pre-harvest sprouting (Knox et al. 2005, 2012; Singh et al. 2014). For vitreousity, mainly phenotypic studies exist (Dexter et al. 1988, 1989; Sieber et al. 2015) and there are to our knowledge no reports about the genetic architecture of this trait. For TKM or grain size in durum wheat a few studies cover the genetic architecture (Sun et al. 2009; Peleg et al. 2011; Blanco et al. 2012). However, these studies were conducted in several different bi-parental populations and thus give only knowledge about a small part of the durum wheat germplasm. Furthermore, the combined investigation of protein content with sedimentation volume, falling number, vitreousity and TKM in Central European durum germplasm is so far lacking.

THE OTHER SIDE OF THE MEDAL – GRAIN QUALITY IN COMBINATION WITH GRAIN YIELD

Undoubtedly, quality traits have a high priority in durum wheat breeding, since farmers need to meet high quality demands to achieve maximum prices. However, a major determinant for their economic turnover is still grain yield (Longin et al. 2013). Thus, a successful variety in the market should combine a good quality profile and a good agronomic performance. Besides the fact that this expands the list of traits that need to be considered in durum breeding programs it can be a great challenge, when there are negative correlations between quality traits and grain yield.

This is the case for protein content, since it correlates negatively with grain yield in durum wheat (Blanco et al. 2006). As mentioned before protein content is of high importance in durum wheat and it is frequently used to price seed batches, since it can be assessed fast and easily (Sieber et al. 2015) and it influences the quality of the final food product (Fois et al. 2011; Kaur et al. 2015). The negative correlation between protein content and grain yield is also well known for bread wheat and it comprises the risk that breeding for higher grain yield will lead to a reduction of the grain protein content (Acreche and Slafer 2009; Würschum et al. 2016).

The protein yield being the product of grain yield and relative protein content was proposed as selection criterion in order to simultaneously improve both traits (Koekemoer et al. 1999). The protein yield corresponds to the harvested grain protein per acreage and it showed a strong positive correlation with grain yield, but only a weak correlation with protein content (Koekemoer et al. 1999). Monaghan et al. (2001) suggested the grain protein deviation (GPD) as an alternate selection criterion. The GPD is defined as the residuals of the regression of protein content on grain yield so that with a high GPD genotypes are identified showing a higher than expected protein content in relation to a given yield level (Oury and Godin 2007). An experiment with 27 bread wheat varieties in multi-environment field trials measured a stable genetic effect over different environments suggesting the GPD as promising selection criterion for breeding (Bogard et al. 2010).

Although first studies about protein yield and GPD indicated their potential merit in wheat breeding, there is not much known about the genetic control of these traits. For grain yield and protein content a large number of studies explored their genetic control in bread and durum wheat, reporting numerous QTL for grain yield (Quarrie et al. 2005; Maccaferri et al. 2008; Bogard et al. 2013; Addison et al. 2016; Mahjourimajd et al. 2016) and for protein content (Blanco et al. 2006, 2012; Maccaferri et al. 2008; Raman et al. 2009; Suprayogi et al. 2009; Brevis and Dubcovsky 2010; Tiwari et al. 2016; Würschum et al. 2016; Dao et al. 2017). This is in accordance with mainly quantitatively inherited traits that tend to be affected by the environment and the population under investigation. However, it is not possible to conclude from these studies on the genetic architecture of the GPD or similarly derived traits.

HEALTH ASPECTS OF WHEAT – HOW PLANT BREEDING COULD CONTRIBUTE TO A REDUCTION OF ACRYLAMIDE

Health aspects associated with the consumption of bread wheat are of major relevance, as wheat is one of the most important staple crops worldwide (FAOSTAT 2019). In general, bread wheat plays an important role for a healthy diet, as supported by medical studies and recognized by several international organizations like the WHO, FAO, and EFSA (cf. Huang et al. 2015).

In the global grading systems of wheat grains, health aspects already play an important role. A prominent example is the contamination of wheat grains with mycotoxins caused by Fusarium. The possible health risk is thereby not related to ingredients coming from wheat and the wheat variety is only indirectly associated with it due to its susceptibility to the fungus (Gaikpa et al. 2019). However, progress in the field of measurement technology and medical research has led to the discovery of several potentially harmful ingredients that were directly linked to wheat. One of those is acrylamide, which is formed in potentially harmful concentrations when cereals such as wheat are treated with high temperatures over a long period during the processing to food products (Claus et al. 2008). Acrylamide was measured in relatively high concentrations in crispbread, cookies, and gingerbread, but it was also found in lower concentrations in bread, bread rolls, and breakfast cereals (Becalski et al. 2003; Svensson et al. 2003; Sadd and Hamlet 2005). Therefore, a number of methods were developed to decrease the formation of acrylamide during the production process. For baked products the reduction of heat, but also a prolonged fermentation time of the dough or the addition of specific ingredients, for example cysteine or asparaginase, have been suggested (Claus et al. 2008).

Another promising strategy would be to decrease the precursors in the raw material and hence reduce the potential for acrylamide formation. Acrylamide forms mainly from carbonyl sources and free asparagine as part of the Maillard reaction (Mottram et al. 2002; Stadler et al. 2002; Zyzak et al. 2003). Since the carbonyl sources are available in excess during the production process of most cereal products, asparagine is the limiting precursor for the formation of acrylamide in cereal products (Surdyk et al. 2004). Thus, most studies with the goal to lower the precursors in the raw material of cereals concentrated on the asparagine content. Experimental field trials with wheat showed that the asparagine content in the grains significantly increased under sulfur-deficient conditions (Shewry et al. 1983; Muttucumaru et al. 2006). Inversely, however, an additional application of sulfur fertilizer in non-sulfur-deprived environments led to no observable decrease of the asparagine content in the wheat kernels (Claus et al. 2006). Experiments based on a smaller number of wheat varieties grown at multiple locations revealed an effect of the variety on the asparagine content, but often also a high effect of the environment (Claus et al. 2006; Corol et al. 2016; Curtis et al. 2018). These studies suggested that variety selection and plant breeding might contribute to a reduction of the asparagine concentrations in the raw material. However, the number of varieties tested in these experiments was low and information about the heritability and the genetic architecture of asparagine content in wheat grown under field conditions was lacking. Only one study utilizing 92 Australian wheat genotypes reported a heritability of 0.32 and analyzed the genetic

architecture of asparagine content (Emebiri 2014). This study identified putative QTL on chromosome 5A explaining up to 24% of the observed variation for asparagine content. However, this study was conducted in greenhouses and it is unclear whether the results are applicable to wheat grown under field conditions.

CONSUMER TRENDS - THE HERITABILITY OF BREAD TASTE

Beyond the wish for a nutritious and safe food product, there is an increasing interest of consumers for high quality products with a focus on exquisite taste. In this context, consumers act to a great extent emotionally. Thereby, a convincing product combines a taste and product story, which serve as vehicle for pleasure, culture and tradition (Barilla Center for Food & Nutrition 2012; Puratos 2012). Considering this, there is a chance to establish attractive niche markets with profitable product chains, especially for small to medium-sized farmers, millers, and bakers (Longin and Würschum 2016).

A good example for this development is the renaissance of spelt (*T. aestivum* ssp. *spelta*) a hulled wheat, which currently receives renewed attention as food and feed grain (Campbell 1997; Longin et al. 2016). Spelt was one of the most important cereals in Southern Germany, Austria and Switzerland between the 12th and 19th century (Gradman 1901) and cultivated in ten thousands of hectares in the United States, too. This changed with the beginning of the 20th century, when the higher yielding and free threshing bread wheat almost entirely replaced spelt (Longin et al. 2016). However, in the last three decades, spelt enjoys growing demand from consumers, bakers, and farmers having led to > 100,000 ha grown especially in Germany and neighboring countries. Consumers associate spelt with tradition, good taste, and a less intensive production and the increasing demand leads to attractive profit margins across the whole product chain. In order to support a sustainable development across the whole product chain of spelt and to satisfy the high consumer demands in the future, information about the agronomic performance, quality traits, and flavor of spelt and its products is of high interest.

In the last decades plant breeding largely contributed to the success story of spelt by introducing genotypes with reduced plant height. Thereby, plant breeding drastically minimized the risk of lodging and increased the yield potential, allowing farmers a profitable production of spelt. Nowadays farmers reach a hulled yield of up to 8 tons per hectare of which about 70% remain as final grain yield after dehulling and separating the chaff from the grain (Longin and Würschum 2014; Longin et al. 2016). Generally, the protein content of spelt is notably higher than that of bread wheat, but the protein quality estimated by the sedimentation volume is different, with lower values for spelt wheat compared to bread wheat (Longin et al. 2016). Nevertheless, Schober et al. (2002) observed a significant genetic variation for bread-making quality between different spelt varieties. However, this study evaluated only very old spelt varieties under a low input farming system. Furthermore, studies investigating beside agronomy and quality also the flavor and odor of the breads are to the best of my knowledge not available in the literature.

OBJECTIVES

Taken together, quality in wheat is a diverse collection of different parameters, which shall ensure an efficient production to obtain a final product with the intended quality. These parameters strongly vary according to the wheat species or the intended target market. They are not set in stone and may change due to new production methods, new legal regulations or a change in consumer demands. The role of plant breeding is to further optimize existing quality and to combine it with a high agronomic productivity. Moreover, plant breeding can take up new developments and contribute to a substantial benefit along the whole production chain. Undoubtedly, this is a great challenge, because of the high number of traits under consideration, which reduce the selection intensity per single trait. In addition, largely influenced by the environment, many of the quality traits underlie a complex inheritance. Thus, knowledge about the genetic architecture of those traits and the potential of genomics-assisted breeding might substantially benefit wheat breeding.

The aim of this thesis was therefore to investigate quality traits in three commercially important wheat species: durum wheat, bread wheat, and spelt wheat. Depending on the species, different quality traits are of major relevance, whereas some as the protein content and its quality are to some extend important for all three species. Thus, we evaluated diverse traits and assessed the potential of plant breeding for their improvement. To assess the potential for genomics-assisted breeding, we placed a special emphasis on the genetic architecture of the evaluated traits.

For durum wheat, our particular interests were to:

1. Determine the genetic architecture of the quality traits protein content, sedimentation volume, falling number, vitreousity and TKM
2. Evaluate the advantages and disadvantages of GPD, protein yield and additional indices for simultaneous improvement of protein content and grain yield
3. Draw conclusions on genomics-aided breeding in durum wheat.

For bread wheat, our special interest was to:

1. Explore the phenotypic variation as well as the heritability for asparagine content and relate it to other important quality traits to assess possible negative effects of breeding for low asparagine content in bread wheat.
2. Moreover, we explored the genetic architecture of asparagine content in order to evaluate the potential of genomic-assisted breeding.

For spelt wheat our objectives were to:

1. Assess the genetic variability and heritability of agronomic and quality traits as well as flavor and odor of the breads.
2. Draw conclusions on breeding spelt cultivars with improved yield, quality and flavor of their end-products.

2 PUBLICATION I: T(A/E)STING SPELT

Spelt: Agronomy, Quality, and Flavor of Its Breads from 30 Varieties Tested across Multiple Environments

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Abstract

The hulled hexaploid wheat subspecies Spelt (*T. aestivum* ssp. *spelta*) is increasingly attracting interest of consumers, bakers, millers, and farmers. Our objectives were to (1) estimate the genetic variability and heritability of agronomic and quality traits in combination with bread flavor and odor, (2) investigate relationships between these traits and (3) evaluate the perspective of spelt breeding focusing on improved yield, quality, and flavor of end-products.

To this end, we evaluated 30 spelt varieties at up to six field sites and assessed important agronomic traits, various quality parameters, and finally bread flavor and bread odor from each variety. Similarly as for the close relative bread wheat, protein and gluten content showed a strong correlation in spelt. However, the two parameters showed just a moderate correlation with protein quality. The correlation of sedimentation volume determined once with sodium dodecyl sulfate (SDSS) and once according to Zeleny (Z-SDS) was very high ($r = 0.94$, $p < 0.001$). However, in the assessed panel the range of sedimentation volume was higher for SDSS and is thus recommended as method for spelt breeding and evaluations. The heritability for bread flavor was 0.56 with an observed significant genetic variation. Moreover, bread flavor was not correlated with other important traits for spelt breeding, such as protein quality and agronomy. Therefore, future spelt breeding could jointly target improved yield, bread-making quality, and a more aromatic bread flavor to be combined in new varieties. This might also be of interest for durum and bread wheat. Our investigations show that considering bread taste as breeding trait is possible, but there is a need of an increased interdisciplinary research to develop faster methods for flavor and odor evaluation of breads.

3 PUBLICATION II: IMPROVING GRAIN YIELD AND PROTEIN CONTENT

Simultaneous improvement of grain yield and protein content in durum wheat by different phenotypic indices and genomic selection

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Abstract

Grain yield and protein content are key traits in durum wheat breeding. However, the negative correlation between those traits has been a constant hurdle for a simultaneous improvement.

To address this in wheat breeding, the grain protein deviation (GPD) and the protein yield were suggested as selection criterions. This research aimed to evaluate the potential of different indices for improving grain yield and protein content in durum wheat. In addition the genetic architecture of those traits and genomics-assisted breeding approaches were investigated. For that purpose, two different durum wheat panels comprising 159 and 189 genotypes, were evaluated at multi-location field trials across Europe and were genotyped by a genotyping-by-sequencing approach. All traits, including the traits grain yield, protein content, and the phenotypic indices showed significant genetic variances and heritabilities in a similar range. The GPD correlated highly and positively with protein content, whereas protein yield showed a high and positive correlation with grain yield. Consequently, a selection based on a high GPD would mainly increase the protein content whereas selecting on a high protein yield would mainly lead to higher grain yields. Nevertheless, a combination of both indices might balance the selection. A genome-wide association mapping indicated a complex genetic architecture for all traits with most QTL having minor effects and being identified only in one germplasm set. This limits the potential of marker-assisted selection for trait improvement. However, genome-wide prediction yielded promising results, but its efficiency strongly depended on the relatedness of training and prediction set.

4 PUBLICATION III: ASPARAGINE IN WHEAT

Assessing the variation and genetic architecture of asparagine content in wheat: What can plant breeding contribute to a reduction in the acrylamide precursor?

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Abstract

During the baking process of wheat products, asparagine and carbonyls sources can form acrylamide a potentially carcinogenic substance. From the perspective of the grains as raw material for baked products the asparagine content is the determining factor in the formation of acrylamide. For this reason, our goal was to assess the potential of lowering the asparagine content of wheat grains by breeding, to contribute to a reduction of acrylamide. Therefor, we analyzed the asparagine content, sulfur content, and five important quality parameters in a set of 149 wheat varieties from Central Europe grown at three different sites. The mean asparagine content of different wheat varieties was between 143.25 and 392.75 mg/kg. These findings reveal that by choosing appropriate wheat varieties the acrylamide content of baked wheat products may already considerably reduced. Moreover, an estimated heritability of 0.65 and no strong correlations with important quality traits like protein content, sedimentation volume and falling number indicate that breeding of wheat with baking quality and reduced asparagine content is possible. Genome-wide association mapping detected few QTL for asparagine content, here the largest QTL explained 18% of the genotypic variance. Using these QTL as fixed effects in a genome-wide prediction resulted in a mean cross-validated prediction ability

of 0.62. Due to a high observed genotype-by-environment interaction for asparagine content, we recommend the expensive and time-consuming laboratory analysis only in later breeding generations. In early generations marker-assisted or genomic selection could serve as valuable tools for selection.

5 PUBLICATION IV: DURUM QUALITY

Evaluation of the genetic architecture and the potential of genomics-assisted breeding of quality traits in two large panels of durum wheat

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Abstract

The global durum wheat (*Triticum turgidum* ssp. *durum*) yield is almost exclusively used for human consumption mainly as pasta and to a lower extent as couscous, bulgur, and further products. Therefore, the demand for quality traits in durum wheat is especially high. In this research, we investigated the quality traits protein content, sedimentation volume, falling number, vitreousity, and thousand kernel mass in a Central European (CP) and a South Western European panel (SP) consisting of 183 and 159 durum lines, respectively. By genome-wide association mapping we assessed the genetic architecture of the investigated traits. Apart from protein content, we observed for all traits QTL explaining a large proportion of the genotypic variance. However, most of the QTL were detected only in one panel. Nevertheless, for sedimentation volume a genomic region on chromosome 1B appeared effective in both durum wheat panels and a BLAST search based on the emmer and bread wheat reference genomes points towards the candidate gene Glu-B3. This inference was supported by the protein subunit banding pattern analyzed with SDS-PAGE gel electrophoresis. Genomic regions on chromosome 7A explained a considerable proportion of the genotypic variance of vitreousity in both panels. One of the detected QTL had a smaller effect on protein content and might be possibly related to the Pinb-2 locus. Within each panel, we obtained high prediction abilities for genomic prediction. Although, the prediction ability dropped drastically when predicting across both panels, the prediction ability remained larger than 0.4 for protein content and

sedimentation volume. This illustrates a high potential for genomics-aided durum breeding if lab and logistical facilities are available.

6 GENERAL DISCUSSION

Especially the seed storage proteins give wheat flour a unique characteristic, which allows the production of a diverse range of food products even with simple technologies (biscuits, crackers, all kinds of breads and pastries, pasta, grünkern, bulgur, couscous, and many more). The natural variation within the wheat genus results in a varying suitability of wheat species or genotypes within one species to be used for different products (Longin and Würschum 2016; Longin et al. 2016). Therefore, bakers, pasta producers and millers have clear visions on the quality of wheat varieties and prefer certain varieties. Furthermore, plant breeders systematically have started to use this variation in order to create new variation, merge and optimize different quality properties or combine them with further plant characteristics to fulfill the requirements of the product chain.

A modern wheat variety needs to perform well for a high number of quality traits, which in addition must be complemented by a good field performance. During the breeding process, the consideration of a high number of traits leads to a reduction in selection intensity for each single trait. In addition, negative correlations between some of the traits further complicate the breeding process. Moreover, new regulations or consumer trends might also make a revision of established breeding objectives or the integration of new breeding objectives necessary.

The aim of this thesis was therefore to investigate quality traits in the three commercially important wheat species durum wheat, bread wheat, and spelt wheat. We evaluated diverse traits and assessed the potential of plant breeding for trait improvement. In order to evaluate the potential of genomics-assisted breeding, I placed a special emphasis on the genetic architecture of these traits. It has to be noted, that in the following I discuss data from different experiments. This shall only serve as a general classification and should not be understood as exact comparisons, which would for several reasons not be valid statistically.

We conducted experiments with two different diverse durum wheat panels. One durum wheat panel (CE-DW, n = 183/189) represented genotypes adapted to Central European climate and the other panel (SWE-DW; n = 159) consisted of genotypes adapted to Southwestern European climate. In a further experiment, a bread wheat panel (BW) consisted of a diverse set of 149 old and modern bread wheat varieties and the spelt experiment was based on a panel (SPW) of 30 spelt varieties.

WHEAT QUALITY ALONG THE PRODUCTION CHAIN

As mentioned before, a wheat variety needs to fulfill a high number of quality traits, which are measured on the raw material (mostly grains, sometimes hulled grains for spelt) or further processed materials (flour, semolina, dough, etc.). The demanded quality traits shall ensure the

utilization of suitable raw material allowing an efficient processing along the whole production chain, resulting in the intended product quality.

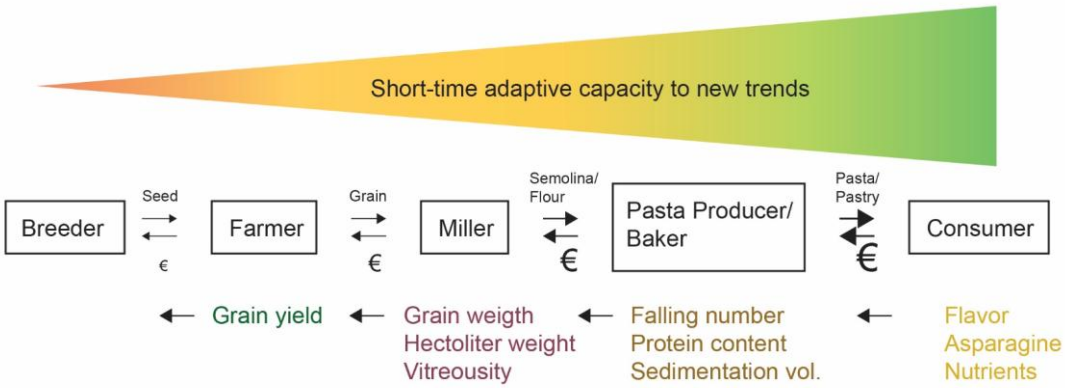

Figure 1 Simplified wheat production chain showing the specific stakeholders. In the upper part illustrating their short-time adaptive capacity to new trends. In the lower part, an extract of important quality traits requested along the production chain is displayed.

At the beginning of the production chain (Fig. 1), preferably easily measurable parameters are assessed, because farmers deliver large seed batches to traders and processors that need to be valued and priced in a short period of time. The next processors, mainly millers, evaluate further quality parameters in order to estimate the need for additives and to compile from different lots a blend with a good cost-benefit ratio and a stable quality asked by the baking sector. With the aid of knowledge and technology, bakers or pasta producers use these blends to produce the final product. The consumer at the end of the production chain finally with his purchasing decision funds these efforts and investments made along the production chain. The purchasing decision is certainly based on main factors like the price, the appearance and the taste of the product, but health aspects and production conditions have become more and more important for consumers in the last years (Barilla Center for Food & Nutrition 2012).

Thus, a wheat breeder is confronted with a number of demands or traits that arise from different levels at the production chain. From a breeder's perspective, traits should preferably allow an easy and rapid assessment, because in a breeding program a high number of genotypes needs to be evaluated in a short period of time, in an ideal case between harvest and sowing of the selected next generation. Hence, most targeted traits are evaluated with quick measuring protocols. A good example of a quick measuring protocol is the determination of the protein content by NIRS already on the combine harvester. In addition, the availability of only a small amount of seeds per genotype in early generations allows only tests, which are non-destructive or need only a small number of seeds. The determination of grain weight is non-destructive and the determination of falling number for instance requires only a few grams of flour. Moreover, some of the traits such as baking quality are expensive to test and thus restricted to a limited number of genotypes. In order to test a high number of genotypes for baking quality, indirect

tests such as the sedimentation volume are important for breeders. Thus, the traits targeted by plant breeders often represent indirect estimators. Due to the long time which is needed to come from a cross to a released new variety, wheat breeders can only react with delay to changing market demands. Thus, they need to anticipate new developments and depend on reliable statements about the necessity of certain quality standards.

THE BASIS OF QUALITY TRAIT IMPROVEMENT

The basis of successful breeding activity is a positive genetic gain. Since the breeding sector is a competitive market, it is important to increase the genetic gain per time so that the actual "breeder's equation" is defined as $\Delta G = \frac{i \times h \times \sigma_G}{Y}$, where i is the selection intensity, h the square root of the heritability, σ_G the genetic variance and Y the length of a breeding cycle. In order to maximize the genetic gain per time, all variables in the numerator should be increased while the length of a breeding cycle should be decreased.

The genetic variance, which is population and trait specific, is the basis of all successful conventional breeding efforts. It might be increased by integrating additional germplasm; however, this needs careful consideration in elite breeding. Plant material that is contributes new variation for some traits but does not belong to the regular breeding pool might have low values in a range of other targeted traits.

In our experiments in all wheat species, we observed a genotypic variance significantly larger than zero ($P < 0.05$, Rapp et al. 2017, 2018a, b, 2019) for all relevant quality traits. Certainly, the existence of variation alone is not sufficient to generate new superior varieties since genotypes below the average are less likely to contribute positively to a trait improvement. Nevertheless, the fact that there was no genotype showing best values for all traits illustrates that there might be potential for improvement within the studied plant materials.

The selection intensity may be increased by selecting a smaller fraction of individuals. However, the selection intensity does not increase linearly to the selected fraction so that in a range of already small selected fractions, the selection intensity increases only marginally (Becker 2011).

The high number of demanded quality traits further limits the selection intensity on a single trait since a balance among all traits needs to be found. Theoretically, with a higher number of targeted traits in a segregating population the number of genotypes that express the best gene combination for all traits should become smaller. However, in practice it is likely that those genotypes do not even exist in the segregating population and one has to find a compromise and lower the selection thresholds for single traits and select more genotypes than is the case when only considering a single trait. In order to maximize a positive genetic gain, this expresses the necessity to keep the number of targeted traits as small as possible.

Surely, the variables in the "breeder's equation" are to some extend interrelated. For example higher selection intensities could lead to a smaller number of selected genotypes, which could then be tested more intensively. More intense testing, which is related to decreasing the measurement error and thus the phenotypic variation, may increase the heritability. Nevertheless, at the core the heritability is in large part determined by the genetic architecture of a trait and is an indicator for the genetic and environmental influence on a trait.

HERITABILITY OF QUALITY TRAITS

The heritability estimates (h^2) vary strongly for the different quality traits. Across all our experiments, the heritability estimates for sedimentation volume were among the highest of all evaluated traits. In the different wheat species, the heritability for sedimentation volume was between 0.88 for bread wheat and 0.94 in the two evaluated durum wheat panels. In contrast but in line with the literature, in all experiments the protein content showed considerably lower heritabilities, which were in a similar range as those for grain yield (Reif et al. 2011; Würschum et al. 2016). This indicates that sedimentation volume is a trait only marginally affected by the environment while protein content is affected to a larger proportion by the environment. A lower effect of the environment is often due to the action of few major genes, whereas a high impact of the environment is often linked to a complex genetic architecture with the action of many genes having small effects.

In order to evaluate the potential of differently calculated yield-protein indices for the simultaneous improvement of grain yield and protein content in durum wheat, we compared their heritability estimates. We observed similar heritabilities for all yield-protein indices ranging from 0.60 for protein yield in the SWE-DW to 0.67 for protein yield and GPD in the CE-DW and SWE-DW, respectively. In the two durum wheat panel these estimates were in a similar range as for the primary traits grain yield (CE-DW: $h^2 = 0.75$; SWE-DW: $h^2 = 0.60$) and protein content (CE-DW: $h^2 = 0.76$; SWE-DW: $h^2 = 0.75$). Thus, from the perspective of heritability none of the selection indices turned out to be advantageous compared to other indices or the primary traits grain yield and protein content.

For the trait asparagine content, we observed a heritability of 0.65, which was considerably higher than reported from an Australian greenhouse experiment (Emebiri 2014). The heritability for asparagine content in our experiment was still of moderate magnitude, but in a comparable range as that for protein content. This suggests that theoretically the response to selection for lower asparagine content in bread wheat could be expected to be similar to that for protein content. However, it has to be noted, that the asparagine content actually requires much more effort to be assessed, which will certainly limit the selection gain in practice. For the relatively unexplored trait bread flavor of spelt wheat, we determined a heritability of 0.56. Compared to the other traits this was at a lower to medium range. However, in combination with the significant genetic variance, it still seems possible to also improve this trait by ongoing breeding efforts.

STEPWISE SELECTION FOR QUALITY

Besides the evaluation of the expected efficiency of breeding efforts for single traits, the heritability is also useful to schedule traits in a stepwise selection for example based on the pedigree-breeding scheme (Fig. 2).

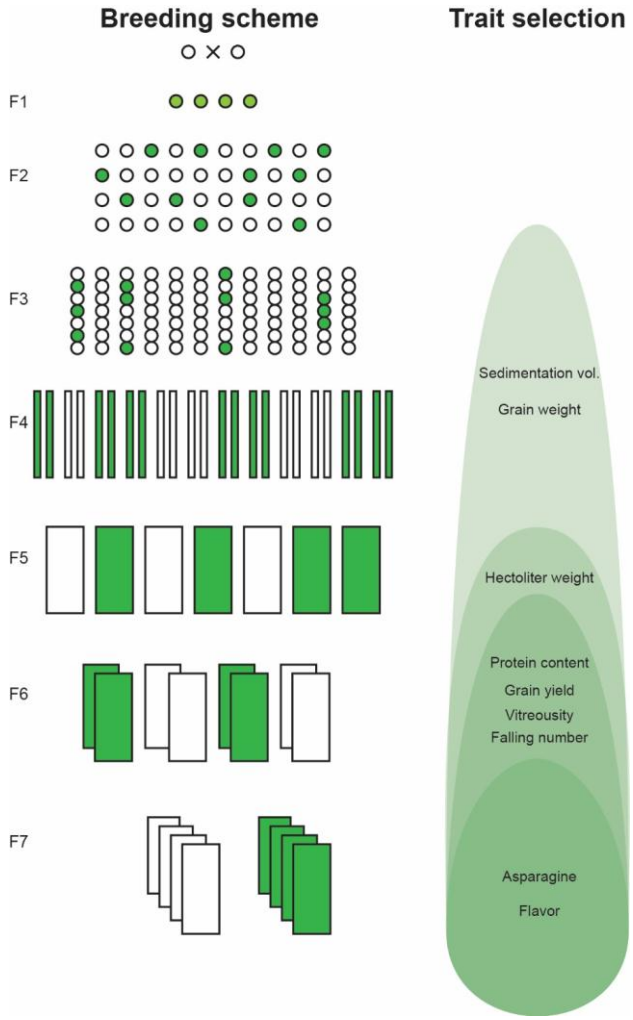

Figure 2 Illustration of a simplified pedigree-breeding scheme, on the right complemented by a suggested step-wise selection for different quality traits in wheat breeding programs.

In this context, also the ratio between genotypic and genotype-by-environment interaction variation as components of the heritability may require additional consideration. In durum wheat the ratio of genotypic variance to genotype-by-environment interaction variance was highest for sedimentation volume with 7.1 in the CE-DW (SWE-DW: 10.8) and lowest for protein content with 1.12 in the CE-DW (SWE-DW: 1.23). These values are in line with reports from bread wheat (Würschum et al. 2016), showing that sedimentation volume was much less affected by interactions between genotype and environment. For asparagine content in bread

wheat, the ratio between the genotypic variance and the genotype-by-environment interaction variance was 1.12, thus, lower than for most other quality traits. This is in line with previous studies that also reported a significant genotype-by-environment interaction for asparagine content and substantiates the strong effect of the environment on this trait(Curtis et al. 2009; Corol et al. 2016).

For traits such as protein content or asparagine content more strongly affected by interactions between genotype and environment, testing should rely much more on field trials carried out in multiple environments. Thus, selection for those traits should be conducted at multiple locations. In a pedigree-breeding scheme, but also other breeding schemes, this is typically the case during later generations (Fig. 2). In contrast, traits that can be reliably tested at fewer environments, like sedimentation or TKM, might allow meaningful phenotypic selection already at early generations.

Certainly, further factors are important for scheduling the selection steps for single traits in the breeding scheme. Among these, the availability of sufficient seed material or adequate testing methods are inevitable. For instance, the bread making quality of wheat genotypes is surely best tested in baking trials. However, besides the high costs for baking trials, they require a larger number of seeds per genotype. Therefore, a direct selection on baking quality is per se only possible in later generations with larger plot sizes per genotype and hence more available seeds (Michel et al. 2018). Thus, for the selection in early generations indirect estimators such as the sedimentation volume need to be utilized. In addition, as indicated before, the costs for assessing the traits play an important role. In the experiment of bread wheat, the determination of asparagine content per sample, constituted a high cost factor. Thus, in breeding programs testing asparagine in early generations with a high number of genotypes does not seems affordable. Unfortunately, the development of a NIRS calibration to be applied as quick and cheap testing method has not yielded a sufficient accuracy yet.

FROM SINGLE TRAITS TO MULTIPLE TRAITS

For the selection on multiple traits, further factors need to be considered. Especially strong negative correlations between targeted traits that should be increased or decreased in parallel poses a great challenge. The selection for best values in one of the traits would imply a selection for poor values in the other trait. Thus, the simultaneous selection on both traits must always balance acceptable values for both traits. For this reason, when considering including a novel trait as breeding target, an interesting question is how this trait is correlated with the established breeding targets. In bread wheat, we were interested in the potential to breed for low asparagine content. Thus, the correlation between asparagine content and other established quality traits represented an important parameter to evaluate the feasibility to include low asparagine breeding in regular breeding programs.

In our experiment, we observed no strong correlation between asparagine content and the other assessed traits, which included the most important quality traits in bread wheat breeding (Fig. 3, p. 21). The sedimentation volume (Z-SDS), as an estimator for baking quality, showed the highest correlation with asparagine content, which, however, was still weak (r = -0.29). A study based on 150 bread wheat varieties grown at one location in Hungary also reported only a weak correlation between asparagine content and Z-SDS (Corol et al. 2016). Thus, the presence of only weak correlations suggests that it is possible to integrate a selection on low asparagine content in breeding programs, without direct negative effects on other evaluated traits.

When looking at correlations, one has to be aware that a correlation is not revealing the causal reason and one has to be careful when drawing conclusion. For instance in the CE-DW, we observed a positive and significant correlation of r = 0.53 (P < 0.001) between vitreousity and falling number. This might lead to the conclusion that both traits are in larger parts influenced by the same grain components. On the other hand, it is also well known that both traits are negatively influenced by rainfalls and wet conditions at grain maturity (Dick et al. 1974; Dexter et al. 1989). Thus, this correlation might also arise from a harvest effect, since all genotypes were harvested at the same time, meaning that genotypes of earlier maturity groups just might have been exposed to more rainfalls at the developmental stage of grain maturity. Indeed, we found a positive correlation between heading time, vitreousity, and falling number, which might indicate such a relationship.

The protein content plays a key role in wheat quality. It is related to some extent to the quality of the end product. For instance, for durum wheat there are reports that the protein content considerably affects the quality of the final food product (Fois et al. 2011; Kaur et al. 2015). Therefore, it is not surprising that more specific quality parameters as the vitreousity in durum wheat (CE-DW: r = 0.37; SWE-DW: r = 0.63; P < 0.001) or the sedimentation volume were to some extent positively correlated with the protein content in most of our experiments (CE-DW: r = 0.02; SWE-DW: r = 0.29; BW: r = 0.16; SPW: r = 0.41/0.52). Nevertheless, on closer examination in most cases in our experiments the correlations were rather weak and not as high as one might have expected.

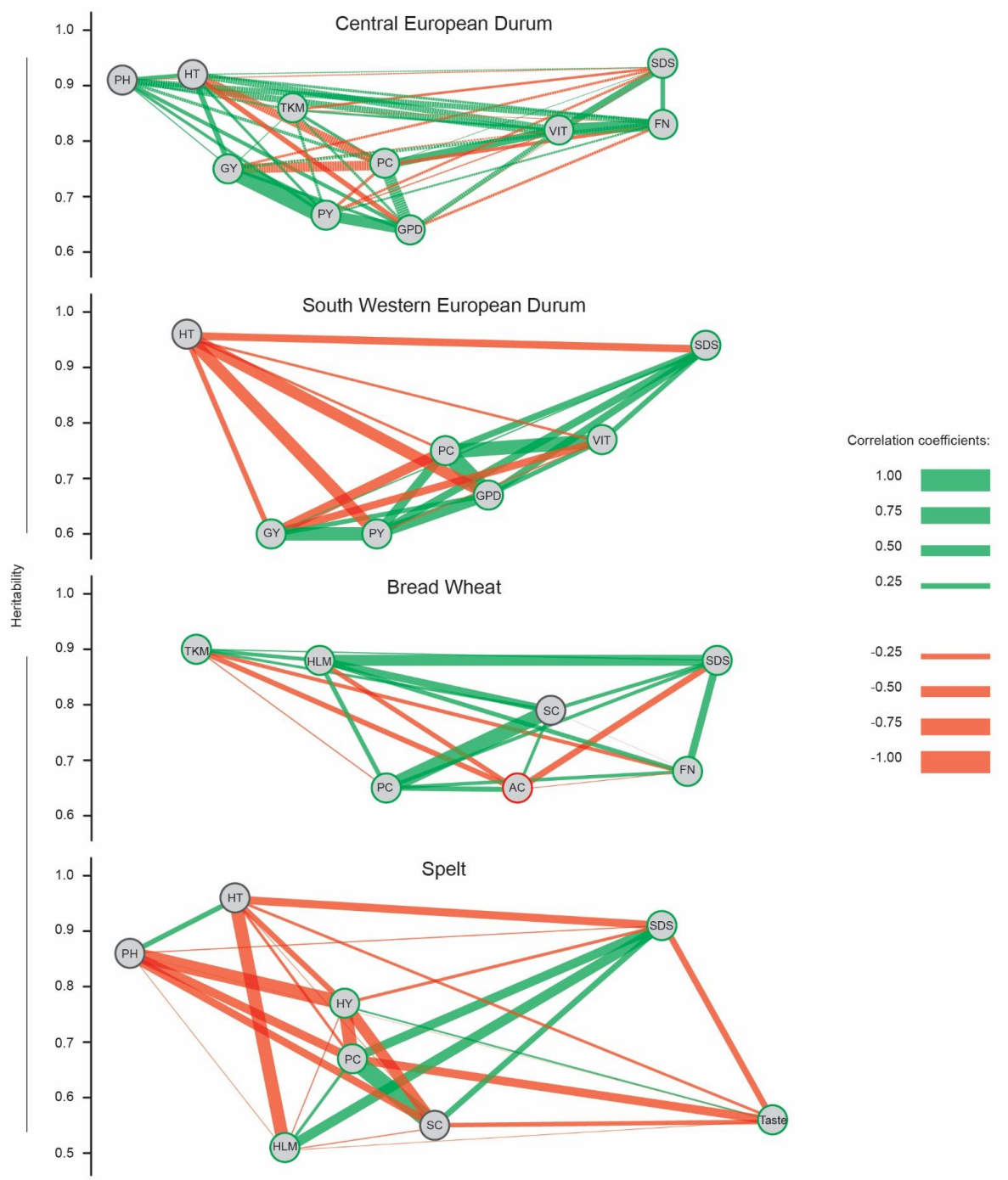

Figure 3 Network plot for different wheat species. Traits are ordered in the vertical dimension according to their heritability. The positive (green) and negative (red) correlation between traits is reflected by the thickness of lines. Dashed lines indicate correlations between traits assessed in different trials. Plant height (PH), heading time (HT), grain yield (GY), thousand kernel mass (TKM), protein yield (PY), grain protein deviation (GPD), vitreousity (VIT), sedimentation volume (SDS), falling number (FN), hectoliter mass (HLM), asparagine content (AC), sulfur content (SC), hulled yield (HY), flavor (FLV).

Moreover, in the baking experiment of 30 spelt genotypes, we observed a positive correlation between dough quality and sedimentation volume, while we could not detect a significant correlation between dough quality and protein content. This was certainly due to the fact that not only the protein content but also its composition determines the final bread quality (Uhlen et al. 2004; Maphosa et al. 2015). In this regard, the question arises whether the high importance of the protein content in grading wheat is justified.

Certainly, part of the popularity of the protein content as quality trait is probably also due to its easy determination by NIRS and the possibility to judge seed batches rapidly. Thus, although the relationship between protein content and quality cannot be indicated in general, it is still an integral part of wheat trading and strongly demanded. A varying positive correlation with related quality traits should also not be a fundamental problem for the general breeding success, it just means that a breeder possibly needs to consider more quality traits.

However, the well-known negative correlation between protein content and grain yield (Blanco et al. 2006; Oury and Godin 2007; Würschum et al. 2016) is a great challenge, especially since grain yield is one of the major breeding objectives in most field crops and of highest importance in wheat breeding. In line with the numerous reports, we observed a significant negative correlation between protein content and yield in all experiments where we assessed both traits. In durum wheat we detected a negative correlation between protein content and grain yield (CE-DW: $r = -0.60$; SWE-DW: $r = -0.45$; $P < 0.001$) and in spelt wheat a negative correlation between protein content and hulled yield (SPW: $r = -0.60$; $P < 0.001$).

Based on the yield experiments of the two durum wheat panels grown at six diverse environments across Europe, we explored the potential of simultaneously improving grain yield and protein content by phenotypic indices. In order to moderate the negative correlation of grain yield and protein content in breeding programs of bread wheat, the grain protein deviation (GPD) was proposed as alternative selection tool (Monaghan et al. 2001). Based on the phenotypic data, we evaluated the usefulness of the selection indices GPD, protein yield, and further indices for durum wheat breeding. Among all yield-protein indices, we determined a high positive correlation in both durum wheat panels, ranging from 0.61 to 1.00, which is reasonable since all indices originate from the same grain yield and protein content data.

The GPD showed a strong and positive correlation with protein content, whereas protein yield was highly and positively correlated with grain yield, which was in line with findings in bread wheat (Thorwarth et al. 2018). Calculating a selection differential based on the 10% best genotypes for each index, revealed that selecting on a high GPD would most likely mainly contribute to an improvement of the protein content. Accordingly, the selection differential for protein yield indicated mainly an improvement of grain yield.

The strong and positive correlation of the GPD with protein content and a significantly weaker positive correlation with grain yield might at least partly be explained by the way of calculating the GPD. The GPD is defined as the residuals obtained from the regression of protein content

on grain yield, representing the distance to the regression line. Thereby the assignment of the protein content as response variable might result in a stronger correlation between GPD and protein content. For example, if there was no correlation between grain yield and protein content, the regression line would be a horizontal line and only the amount of the protein content would account for the resulting GPD values. Thus, with a weaker correlation between grain yield and protein content, a stronger correlation between GPD and protein content can be expected and consequently a stronger selection on protein content.

In order to adjust for the strong correlation with protein content, we used a similar approach as for the GPD to calculate a yield deviation. However, in this case we switched the response and explanatory variable so that we calculated the regression of grain yield on protein content. In fact, this led to an index, which showed a strong and significant correlation with grain yield and only a marginal correlation with protein content. We finally combined the GPD and yield deviation in a further index by forming the sum of their standardized values ($index_{GP\&YD}$). This $index_{GP\&YD}$ was to a similar extent correlated with grain yield and protein content. Compared to all other indices, the $index_{GP\&YD}$ appeared to deliver the most balanced selection differentials allowing a parallel improvement of both primary traits grain yield and protein content to a similar extent. In line with this recent, simulations about the expected response to selection with closely related indices came to similar conclusions (Michel et al. 2019).

GENETIC ARCHITECTURE OF TRAITS

Genome-wide association mapping has proven to be a valuable tool to examine the genetic architecture in diverse plant material sets (Reif et al. 2011; Würschum et al. 2011) Therefore, genotypes were genotyped by a genotyping-by-sequencing approach. After quality checks 12,086 and 10,505 polymorphic markers with known map position were available for the CE-DW and SWE-DW, respectively. While for bread wheat the marker set consisted of 22,122 polymorphic markers with known map position (Li et al. 2015).

For the different traits, which we assessed in durum wheat and bread wheat by genome-wide association mapping, we observed a large number of putative QTL. Most of the detected QTL explained only a small proportion of the genetic variance. Nevertheless, for some of the traits we were able to detect medium- to large-effect QTL. Among all traits, the strongest marker-trait association was observed for sedimentation volume in the two durum wheat panels. The putative QTL detected in each panel were located in a similar genomic region on chromosome 1B. They explained a proportion of 49.5% and 36.9% of the genotypic variance in the CE-DW and SWE-DW, respectively. In wheat, several genes coding for gluten proteins are located on chromosome 1 of the different subgenomes (Payne 1987). The resulting gluten proteins can be divided into the main groups glutenins and gliadins (Wieser 2007).

By means of a Basic Local Alignment Search Tool (BLAST) approach, we were able to assign most of the markers of chromosomes 1A and 1B and some of the glutenin and gliadin genes a physical map position according to the reference genome of wild emmer wheat and bread wheat (Avni et al. 2017; CNR InterOmics 2017; IWGSC RefSeq v1.0; Alaux et al. 2018). This revealed the *Glu-3*, *Gli-1*, and *Gli-3* genes as being physically close to the detected main QTL regions on chromosomes 1A and 1B. This is in line with recent studies detecting QTL for sedimentation volume in the proximity of the *Glu-3* and *Gli-1* loci in North American durum wheat populations (Kumar et al. 2013; Fiedler et al. 2017). Payne et al. (1984) concluded that rather the low molecular weight glutenins should have the main effect on sedimentation volume in durum wheat and not gliadins, because gliadins are freely soluble and unlikely to have a measurable effect on sedimentation volume. Consistently, two different studies on durum wheat observed a particularly high influence of the low molecular weight glutenins encoded at the *Glu-B3* locus on gluten strength (Brites and Carrillo 2001; De Santis et al. 2017). Thus, based on our findings and reports in the literature the *Glu-B3* locus appears to have the strongest effect on sedimentation volume and gluten strength in durum wheat.

These findings are in contrast to reports from bread wheat. A genome-wide association mapping for sedimentation volume in bread wheat was not part of our studies. However, a recent study in bread wheat reported that allelic variants at the *Glu-A1*, *Glu-B1*, and *Gli-B1* loci, explained large proportions of the genotypic variance of sedimentation volume (Würschum et al. 2016). Thus, different to durum wheat, in bread wheat variation for high molecular weight glutenins seems to have a stronger effect on sedimentation volume.

In contrast to the sedimentation volume, for protein content in the durum wheat experiments we only observed QTL explaining a smaller proportion of the genotypic variance. This is in line with previous reports about durum and bread wheat describing protein content as a quantitatively inherited trait with many QTL contributing only a small proportion to the genotypic variance (Blanco et al. 2006, 2012; Suprayogi et al. 2009; Würschum et al. 2016; Fiedler et al. 2017; Thorwarth et al. 2018). However, in durum wheat we observed a concentration of detected putative QTL for protein content on chromosome 7A. This was similar for vitreousity in durum wheat in the two panels, although a main peak of significant marker-trait associations on chromosome 7A between 56.6 and 75.6 cM appeared to be independent from protein content. Moreover, the putative QTL that with 35.6% explained the largest proportion of the genotypic variance was only detected in the CE-DW. The marker identifying this main putative QTL seemed to be almost fixed in the SWE-DW and its small positive effect might be due to this low allele frequency. Even though the effect was rather small, we identified a putative QTL in the SWE-DW and CE-DW for vitreousity and for protein content in a consistent region of chromosome 7A. Thus, this putative QTL might represent a genomic region causing the positive correlation between protein content and vitreousity observed in this and a previous study (Sieber et al. 2015). Whether this putative QTL traces back to a single gene with pleiotropic effect on both traits or to closely linked genes for protein content and vitreousity warrants further research. On chromosome 7A the *Pinb-2* gene is associated with the trait grain hardness (Wilkinson et al. 2008), that in turn correlates to a certain extent with grain vitreousity (Dexter et al. 1988). Indeed, the analyses of the physical distance of the putatively common QTL for protein content and vitreousity and *Pinb-2* (Chen et al. 2010) revealed a relatively close physical distance. Hence, the *Pinb-2* gene is a likely candidate for the putative protein content and vitreousity QTL detected on chromosome 7A.

For TKM and falling number that we investigated only in the CE-DW, we identified one putative medium- to large-effect QTL, respectively. The putative main QTL for falling number was found on chromosome 7A and explained 21.0% of the genotypic variance. In combination with the *Wx-B1* gene, the *Wx-A1* gene on chromosome 7A is known to have an effect on the α-amylase activity in durum wheat (Vignaux et al. 2004). Indeed, the trait falling number indirectly estimates the α-amylase activity. When we compared the physical position of the significant marker and the *Wx-A1* gene, the distance was 32.9 Mbp according to the bread wheat reference genome (IWGSC RefSeq v1.0, Alaux et al. 2018). Thus, the relatively large distance does not substantiate a possible genomic accordance between the detected putative QTL for falling number and the *Wx-A1* gene. However, the detected putative QTL might also not directly be linked to the *Wx-A1* locus, but to a more distant regulatory region of the *Wx-A1* gene.

The major putative QTL for TKM was located on chromosome 2A and explained 35.6% of the genotypic variance in the CE-DW. The cell wall invertase gene *TaCwi* (Ma et al. 2012; Jiang et al. 2015) on chromosome 2A was reported to affect TKM in bread wheat, but it is not located in proximity (> 350 Mbp) to the detected putative QTL of our study. Similarly, two different QTL for TKM, identified on chromosome 2A in studies on bread wheat were located in great

distance (> 500 Mbp) (Sun et al. 2009; Würschum et al. 2018). In a population derived from a cross between durum wheat and wild emmer a grain weight QTL on chromosome 2A was detected (Peleg et al. 2011), but in comparison to our study the chromosomal regions seemed to be different. With respect to reports in the literature, the highest positional accordance of about 30 Mbp was observed between the putative TKM QTL of our study and a grain size related QTL reported by Breseghello and Sorrells (2007). This QTL (Xbcd1688) was detected in a hexaploid wheat mapping population for grain area, which was strongly correlated with grain weight. However, as mentioned before, based on genomic proximity it cannot be deduced unambiguously whether the two QTL have their origin in an identical genomic region. Nevertheless, this genomic region seems to be a promising target for further research in order to identify a novel grain weight related gene.

In bread wheat, we focused our genome-wide association mapping mainly on asparagine content. Here, we detected eight putative QTL for asparagine content, which jointly explained 78.5% of the genetic variance. A putative QTL on chromosome 7B explained 18.4% of the genetic variance, which was the highest proportion of explained genetic variance by a single marker. The identified QTL for asparagine content in our study were not in line with those published in the Australian study (Emebiri 2014). However, strong differences in the environmental conditions might have led to the contradicting results, especially since the Australian study was carried out in a greenhouse, compared to multi-location field trials in our experiment.

For grain yield that was assessed in durum wheat in connection with the protein content, we mainly detected putative QTL with small effects, each explaining only a small proportion of the phenotypic variance. This is in line with other reports for durum wheat (Maccaferri et al. 2008; Blanco et al. 2012; Soriano et al. 2017) and is typical for a mainly quantitative trait, where the trait expression is influenced by many small effect QTL and by environmental factors.

Comparing putative QTL for grain yield or protein content with QTL detected for the derived yield-protein indices revealed similar putative QTL for some traits. This reflects the phenotypic relatedness or strong correlation of these traits. A similar genetic basis of derived traits and primary traits was also reported for maize (Wang et al. 2012). In general, our data clearly suggest that the genetic architecture for the derived yield-protein indices is similarly quantitative than that of the primary traits grain yield and protein content. Rather, we found a slightly higher number of putative QTL for the primary traits than for the mathematically derived traits. This is in line with reports of Wang et al. (2012) intensively investigating the impact of mathematically deriving traits from primary traits in maize. They concluded that mathematically derived traits had a lower detection power and a higher false discovery rate than their primary traits. Consequently, combining quantitative traits in indices will most likely not facilitate the detection of marker-trait associations.

GENOMICS-ASSISTED BREEDING

The utilization of molecular marker information has the potential to enhance significantly the efficiency of plant breeding programs. The potential benefit of utilizing markers in breeding programs is that it allows replacing costly and time-consuming phenotyping steps. Thereby it may save not only time and costs needed for phenotyping but also capacities may become available to test more genotypes or test only genotypes with desired marker profiles more intensively. In principle, there are two different methods: either using marker-assisted selection (MAS) based on few markers, which are linked to genes affecting the traits of interest, or the application of genomic prediction using a high number of unspecific markers with a whole genome coverage.

POTENTIAL FOR MARKER-ASSISTED SELECTION

Substantially lower costs arise when employing only few markers, based for instance on the KASP technology, which costs less than one Euro per marker and genotype. Importantly, such single markers must explain a high proportion of genotypic variance robustly across different breeding cycles and genetic backgrounds (Bernardo 2008). Based on our results in durum wheat, the development of KASP markers for the *Glu-B3* locus and the QTL detected for TKM might be interesting for a routine application. Certainly, the benefit of marker-assisted selection increases when the phenotyping costs are high or phenotyping of specific traits is not possible due to a lack of enough available seed grains in the respective generations, mainly at early breeding generations.

In bread wheat for instance, we detected a putative QTL for asparagine content on chromosome 7B explaining with 18.0% the highest proportion of genotypic variance among all detected QTL. This QTL appears of minor importance in comparison with detected major QTL in durum wheat for sedimentation volume or TKM explaining up to 49.5% and 35.6% of the genotypic variance. However, the determination of asparagine content is by far much more laborious, time-consuming and costly so that this QTL might still be interesting for the application of marker-assisted selection, especially in early generations.

VALIDATION OF QTL

In general, it has to be noted that before a routine application in MAS, QTL detected in one experiment require validation in further environments with possibly more or different genotypes (Bernardo 2008). Certainly, it is advisable to conduct the phenotyping of QTL surveys in the target growing areas, especially for traits like asparagine content showing a considerable impact of the environment.

In durum wheat, based on the three experiments we were able to compare findings of our QTL analysis in two different panels consisting of different germplasm. For the predominantly

quantitatively inherited traits grain yield, protein content, and derived yield-protein indices we were not able to identify clearly consistent QTL in both panels. Nevertheless, we fitted the significant markers only identified in one panel, in a linear model based on data from the other panel. In the other panel these markers showed low, close to zero, or even low adverse effects. This suggests that the detected QTL of one panel are only effective in this panel. A possible explanation might be a different genetic background of genotypes in the two panels. On the other hand, the two panels were grown in different environments and it is possible that the QTL effects are environment specific (Bernardo 2008).

During the analysis of further traits such as the sedimentation volume, which is much less affected by the environment, we identified most likely common major QTL linked to the *Glu-B3* gene in both panel. Although there were overlapping significant markers between the two panels, the most significant markers identifying those QTL were also in this case different in the two panels. When the marker effects in the other panel were estimated by fitting a linear model, also here we observed a decrease in the proportion of the explained genetic variance by the markers detected in the other panel. However, the decrease was much smaller and the markers of one panel still explained a considerable proportion of the genetic variance.

If we assume that the markers identify the same causal QTL this observation might be attributed to different allele frequencies of markers or different linkage structures between markers in the two panels. However, based on the marker data, this would require the development of panel-specific markers for marker-assisted selection. A possible alternative might be to focus in future research on possible candidate genes, as the suggested *Glu-B3* gene. Resequencing of those genes might lead to the identification of superior alleles and the development of allele-specific functional markers could increase the benefit of marker-assisted selection.

LIMITATIONS OF ASSOCIATION MAPPING

A further aspect that has to be considered in the context of detected QTL explaining a large proportion of the genetic variance is the frequency of desired alleles in the evaluated germplasm. For sedimentation volume, we detected a QTL on chromosome 1B, which explained a large amount of the genetic variance and in the CE-DW genotypes carrying the favorable allele had on average a 40.4% higher sedimentation volume. This suggest that especially for this trait a significant trait improvement might be realized by marker-assisted selection. However, in this case we observed a high rate of the favorable allele for this QTL in the examined plant material. Also for other traits, larger effect QTL in durum wheat showed a high rate of favorable alleles. This might limit the usefulness of the QTL for marker-assisted selection, because most of the elite lines are already fixed at these loci. Hence, in the majority of possible crosses a marker identifying this QTL would not be necessary. This example might illustrate that continuous selection on the sedimentation volume has already led to a fixation of the positive allele in a large number of varieties.

With respect to this, a potentially higher benefit of marker-assisted selection could be expected for traits that were not intensively targeted by breeding in the past. In our experiments, this was the case for the asparagine content in bread wheat. Indeed, in the association mapping the QTL, which explained the highest proportion of the genotypic variance, had a frequency of 0.44 for the favorable allele. Such QTL would be more attractive for marker-assisted selection compared to QTL for which the favorable allele is carried by a high number of genotypes.

However, there might still be a useful application for marker-assisted selection of major QTL showing a high abundance of the favorable allele in elite material. Such markers might be useful when wide crosses are performed with genetic resources or exotic material with unknown or poor quality. For instance, this could be worthwhile for the introgression of disease resistance genes. Here established molecular markers for important quality traits could be used in a marker-assisted background selection in early segregating populations to avoid selecting lines with undesired quality profile. Thus, a good portfolio of established molecular markers for quality traits and agronomic traits might be of high value. It could lead to a less restrained application of exotic material and genetic resources in elite breeding as it might help to contain their unfavorable characteristics.

At last, it has to be remarked that association mapping studies comprise a principle drawback with regard to detection of major but rare QTL. As mentioned before, such QTL are expected to have the largest beneficial effect in marker-assisted selection. In order to control for the rate of false positives it is necessary to exclude markers with a very low minor allele frequency (Kearsey and Farquhar 1998). Thus, due to statistical reasons it is not possible to detect very rare alleles (in our experiments alleles with a minor allele frequency < 0.05) in a diversity panel by means of association mapping, even if the rare alleles have a huge effect on the trait expression (Myles et al. 2009). This implies that it could be worthwhile to re-examine genotypes with an especially good phenotypic performance, which is not attributable to major QTL detected in the association mapping. For these cases, a QTL-mapping study in a generated bi-parental population might be a valuable option.

POTENTIAL FOR GENOMIC SELECTION

In our genome-wide association studies in durum wheat and bread wheat, for most traits we detected a large number of QTL that as single markers explained only a minor proportion of the genetic variance. This is typical for a predominantly quantitative inheritance of traits (Bernardo 2008), which clearly limits possible applications of single markers in marker-assisted selection.

Genomic selection is presented in recent reports as a highly promising approach to boost genetic gain in elite breeding also or especially for quantitatively inherited traits (Guzman et al. 2016; He et al. 2016; Marulanda et al. 2016; Michel et al. 2016, 2018, 2019). In addition to the genome-wide association mapping, we therefore estimated the genomic prediction ability

for all traits applying ridge regression BLUP (RR-BLUP). Based on the bread wheat experiment for the traits asparagine and sulfur content, we evaluated further genomic prediction models including Bayesian and reproducing kernel Hilbert space (RKHS) approaches. The prediction abilities were in a comparable range for all different model approaches, which confirms the RR-BLUP model as well-suited tool for plant breeding, also because of its relatively low demands for computing power.

In durum wheat, the mean cross-validated genome-wide prediction ability for grain yield was 0.46 and 0.51 within the CE-DW and SWE-DW, respectively (Fig. 4). For protein content, the prediction ability was slightly lower with 0.46 and 0.40 for the CE and SE panel, respectively. In bread wheat, the prediction ability was 0.43 for protein content. These results are in line with studies of bread wheat and durum wheat reporting a prediction ability on a similar level for grain yield (He et al. 2016) and protein content (Liu et al. 2016; Würschum et al. 2016; Fiedler et al. 2017). For durum wheat, the prediction ability of protein yield, GPD, yield deviation, and further computed yield-protein indices reached a comparable level as that of grain yield and protein content. This once more indicates, that the computed yield-protein indices had a comparable trait architecture to the primary traits grain yield and protein content.

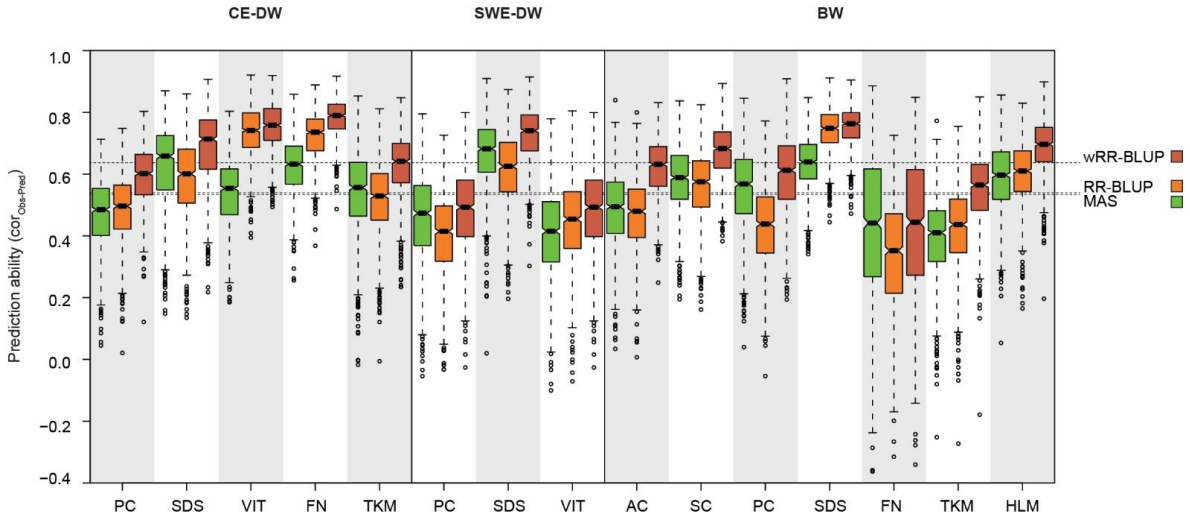

Figure 4 Cross-validated prediction ability of marker-assisted selection (MAS) based on detected QTL explaining more than 10% of genotypic variance and genomic prediction using ridge-regression BLUP (RR-BLUP) or weighted ridge-regression BLUP (wRR-BLUP) for Central European durum wheat (CE-DW), South Western European durum wheat (SWE-DW), and bread wheat (BW). Dashed lines indicate the overall average prediction ability for the three approaches. The explored quality traits were protein content (PC), sedimentation volume (SDS), vitreousity (VIT), falling number (FN), thousand kernel mass (TKM), asparagine content (AC), sulfur content (SC), hectoliter mass (HLM).

For further assessed quality traits in durum wheat, we observed prediction abilities in a range between 0.44 and 0.73. Interestingly, in this case the lowest and highest values were both for

vitreousity in the SWE-DW and CE-DW, respectively. This might indicate a differing gene action in the two experiments possibly caused by different environmental effects. We observed consistently high prediction abilities for sedimentation volume in durum wheat with 0.59 and 0.62 in the CE-DW and SWE-DW, respectively. In line with this, we detected with 0.74 the highest prediction ability for sedimentation volume in our bread wheat experiment. Taken together, our results showed a good accordance with literature reports for durum and bread wheat (Würschum et al. 2016; Fiedler et al. 2017; Haile et al. 2018). For asparagine content in bread wheat, for which no literature reference was available, we observed a prediction ability of 0.58, which is in a similar range as for other genetically complex traits.

The prediction ability for marker-assisted selection based only on the QTL explaining more than 10% of the genotypic variance, was particularly high for sedimentation volume in durum wheat. This might be due to the detected major QTL, which explained a large proportion of the genotypic variance. However, the simulated prediction ability for marker-assisted selection for the other traits also yielded on average only slightly lower average prediction abilities than those obtained by the RR-BLUP approach. In the literature it was shown that for genomic prediction already a smaller marker set was sufficient to achieve a robust prediction ability (Haile et al. 2018). Since much of the prediction ability is linked to the relatedness between individuals an explanation could be that this might be predictable already with a smaller number of markers.

On average, a considerably higher prediction ability was obtained when combining information of identified QTL with genomic selection in a weighted RR-BLUP (wRR-BLUP) in all our experiments. This confirms results of many previous studies indicating the usefulness of incorporating information of genome-wide association mapping in genomic prediction models, in order to increase the prediction ability (Boeven et al. 2016; Würschum et al. 2016; Akel et al. 2019). Simulation studies on optimum breeding schemes showed a significant increase of the annual response to selection when genomic prediction was included in the breeding scheme (Marulanda et al. 2016). Importantly, this applied already at levels of prediction abilities that were reached for all traits in all our experiments.

However, the prediction ability of genomic selection highly depends on the relatedness of the germplasm used as training and prediction sets (Crossa et al. 2014; Fiedler et al. 2017; Haile et al. 2018). We therefore used the CE-DW as training set to predict the traits in the SWE-DW and vice versa. As expected, the prediction abilities of genomic selection dropped considerably when compared to predictions within the same panel. However, while the drop was especially high for grain yield and vitreousity, the decrease was not as strong for protein content and sedimentation volume and the prediction abilities were around 0.4 or higher. This confirms on the one hand once again the importance of the design of training and prediction sets. On the other hand, prediction abilities > 0.4 across different panels appear very interesting to boost the genetic gain per unit time (Marulanda et al. 2016), especially for traits with high environmental impact requiring multi-location phenotyping (e.g. protein content) or for traits cumbersome to measure requiring milling of kernels prior to lab analyses (e.g. sedimentation volume).

IMPLEMENTATION OF GENOMIC SELECTION

In my opinion, the consideration to deploy marker-assisted or genomic selection in a breeding program depends on several preconditions. Firstly, utilizing molecular markers requires a number of capabilities. This includes dealing with the required logistics regarding tissue sampling and tracing of selected lines in the greenhouse or the field within the required timeframe so as not to miss a breeding season. This might not be a problem in large and well-resourced breeding programs mainly in major staple crops. However, in durum wheat or even more so in spelt wheat this might not be realizable in a number of breeding programs around the world in the near future. Second, the costs for genomic selection differ largely across companies and service providers, but are still considerably high. Nevertheless, the genotyping costs per genotype are more or less fixed and can even be expected to further decrease in the future; in addition, the training set that needs to be phenotypically evaluated consists of only a small part of the total breeding material. Thus, with increasing costs for phenotyping, the economic advantage is increasingly shifted towards genomic selection. High phenotyping costs may arise for traits like grain yield or protein content showing a large genotype-by-environment interaction, since they require evaluation in multi-location field trials. In addition, phenotyping is costly for traits like asparagine content that currently needs to be determined through expensive laboratory methods. Moreover, high costs for phenotyping simply add up when a high number of traits must be evaluated. For example, this is the case when assessing the complete phenotypic quality profile of a durum genotype.

Thus, the cost-benefit ratio of genomic selection strongly depends on the focus and the layout of the breeding program and therefore needs regular reassessments due to the potential development of cheaper and high-throughput phenotyping technologies. An advantage for breeding programs already using genomic prediction tools is that it demands a smaller extra effort to include new traits such as asparagine content, in the program, since the new traits need to be assessed only in the training set.

FUTURE CHALLENGES IN BREEDING FOR WHEAT QUALITY

One of the reasons for the global success of wheat has surely always been its unique quality. In an ongoing process, plant breeding has refined the quality and has released a high number of varieties adapted to a range of different applications. It should be stressed, that this was only possible with high and continuous investments in research and in large breeding programs. The importance of plant breeding to provide new adapted varieties can be expected to even increase, since new regulations for the application of fertilizer and increasing weather extremes might considerably shift the general conditions of wheat production (Olesen et al. 2011; Trnka et al. 2014). In order to ensure in wheat high and stable grain yields combined with a good quality in the future, it is necessary for breeding programs to be adequately funded. The best basis therefore is surely the successful selling of seeds. However, this is often stagnating since farm-saved seeds are used to till a large proportion of the wheat growing areas.

Hybrid varieties, which enable maximum yields only in the first generation, are therefore a tempting option for plant breeders. Nevertheless, the implementation of hybrid breeding in all wheat species is linked to a number of obstacles, which need to be solved. This applies to the biology of pollination (Boeven et al. 2016; Akel et al. 2019) but importantly also, the expression of quality in heterozygous hybrid wheat plants is not fully understood. A closer connection along the production chain could also help to increase the selling of seeds, when traders or millers demand new varieties or promote the selling of seeds within cultivation contracts.

An improved communication and interlocking along the value chain could also increase the recognition of the importance of plant breeding and the existing opportunities. Conversely, wheat breeders could profit from a clear communication with the manufacturing sector. In particular, the communication about the actual importance of a quality trait might in the end lead to a reduction of the number of first priority quality traits. This could then facilitate the breeding for the remaining quality traits and increase the response to selection.

In this context, research can serve as mediator between plant breeders and the manufacturing sector. On the one hand, research can estimate the potential of plant breeding, especially for scarcely explored traits such as asparagine content. On the other hand, research may critically review correlations between traits and stimulate the discussion about thresholds or the actual necessity of some quality traits. For example, it is an open question whether the high rating of the protein content is sufficiently reasoned.

The implementation of new genomics-assisted breeding technologies is a great opportunity for plant breeders in general. We could show that genomic selection has a great potential to boost wheat quality breeding. However, for smaller companies the implementation of such new technologies means a great challenge. Collaborations between smaller breeding programs might be a way to afford the necessary investments.

Smaller markets as for example that of spelt wheat offer an additional economical niche for smaller breeding companies, since these markets with smaller profit margins may not be attractive for larger companies. Wheat breeders active in such markets should maintain or promote the specific characteristics of that crop, since it stimulates the interest of consumers. This will again require a good exchange with the manufacturing sector, which in such markets should also have a strong interest in a high product quality.

CONCLUSIONS AND OUTLOOK

Quality is of high importance in all three investigated wheat species. Surely, the quality demands differ between durum wheat, bread wheat, and spelt wheat. Nevertheless, we observed a significant genetic variation for all relevant quality traits. The wide range of variation and moderately high heritability estimates suggested that a breeding success seems possible even for to date neglected traits as the asparagine content in bread wheat or the bread flavor in spelt wheat. Moreover, this conclusion is supported, since we observed no strong negative correlations with a number of other important traits. However, high cost to test for asparagine content and bread flavor highlight the need of an intensified interdisciplinary research to develop faster and cheaper methods to assess these traits. In addition, the initiation of breeding programs for such unexploited traits requires an increased interest of the manufacturing sector, which is normally reflected in a financial benefit for the breeder/farmer.

A simultaneous improvement of the two important but negatively correlated traits grain yield and protein content by means of an index seems possible. However, its efficiency largely depends on the weighting of the single traits. The selection for a high grain protein deviation would mainly increase the protein content whereas a selection based on protein yield would mainly improve grain yield, but a combination of different indices allows balancing this selection.

In the genome-wide association mapping in bread wheat and durum wheat, we were able to detect a large number of putative QTL. This indicated a rather complex genetic architecture for most traits. This also applied to the calculated yield-protein indices. However, in durum wheat we identified putative QTL explaining a large proportion of the genotypic variance for the different traits. For sedimentation volume, a genomic region on chromosome 1B appeared important. A BLAST search against the reference genomes of emmer and bread wheat revealed the *Glu-B3* gene as a likely candidate. For vitreousity, genomic regions on chromosome 7A explained a larger proportion of the genotypic variance in the two explored durum wheat panels, whereas one QTL, possibly related to the *Pinb-2* locus, also slightly influenced the protein content. For TKM we detected a putative QTL, which explained a large proportion of the genetic variance, which probably traces back to a yet unknown gene.

Thus, there is certainly a potential for some of the identified QTL to be used in marker-assisted selection. Furthermore, in a five-fold cross-validation genomic selection was confirmed as a further promising selection tool. We observed average prediction abilities that were on a promising level to increase the selection gain in breeding programs. However, highlighting the importance of relatedness for genomic prediction, the prediction ability dropped considerably when predictions were performed across two different durum wheat panels. Taken together, the decision to use single markers and genomic selection highly depends on the respective breeding program and the underlying germplasm. If logistically possible, I would suggest a breeding method applying first single diagnostic markers and then genomic selection prior to expensive field trials for yield and quality.

7 SUMMARY

Quality traits in wheat are of great importance, as they are required for the production of a wide range of food products. In Europe, bread wheat (*Triticum aestivum* ssp. *aestivum*) for human consumption is primarily used in pastries. For durum wheat (*Triticum turgidum* ssp. *durum*) that is used almost exclusively for pasta production, quality traits are at least as important as in bread wheat. In Central Europe, the bread wheat subspecies spelt (*Triticum aestivum* ssp. *spelta*) is characterized by a different quality compared to bread wheat. In addition, it is produced for a niche market with a particular focus on the final product quality. The high number of demanded quality traits of a wheat variety represents a great challenge for wheat breeders. Thus, knowledge about the genetic architecture and interrelation of quality traits is of high value for wheat breeding. Due to the long list of quality traits in wheat, we focused on currently important quality traits in each of the three wheat species.

In durum wheat, I was interested in traits with a high importance for durum millers and pasta producers. The protein content and the sedimentation volume are of high importance for pasta producers as they influence the firmness of cooked pasta, better known as “al dente”. A low falling number may lead to brown instead of light yellow pasta, which goes back to an increased maillard reaction during pasta production and drying. The vitreousity, representing the glassy appearance of durum grains, and the thousand kernel mass influence the semolina yield and are therefore of great interest for durum millers. In the genome-wide association mapping, I identified several putative QTL for these quality traits. For the sedimentation volume, a genomic region on chromosome 1B appeared to be important. A BLAST search against the reference genomes of emmer and bread wheat revealed the *Glu-B3* gene as a likely candidate. For vitreousity, genomic regions on chromosome 7A explained a larger proportion of the genotypic variance. One of these QTL, possibly related to the *Pinb-2* locus, also slightly influenced the protein content. Thus, this genomic region might be a genomic reason for the positive correlation between vitreousity and protein content. For TKM we detected a putative QTL, which explained a large proportion of the genetic variance, but could not be attributed to a known gene. Besides a good performance for quality traits, a modern durum wheat variety should be complemented by a good agronomic performance, in particular a high grain yield. This poses a great challenge for plant breeders, since grain yield and protein content are negatively correlated. With regard to simultaneously improving grain yield and protein content, the protein yield or the grain protein deviation (GPD) were proposed. We evaluated those and further selection indices for their potential to be utilized for the simultaneous improvement of grain yield and protein content. Our results indicated that a simultaneous improvement of the two traits grain yield and protein content by means of an index seems possible. However, its efficiency largely depends on the weighting of the single traits. The selection for a high GPD would mainly increase the protein content whereas a selection based on protein yield would mainly improve the grain yield. Nevertheless, a combination of different indices allows balancing this selection. Compared to the primary traits grain yield and protein content, the selection indices did not essentially differ in the complexity of their genetic architecture.

In bread wheat, we focused on the acrylamide precursor asparagine. Acrylamide is formed in potentially harmful concentrations when cereals are treated with high temperatures over a long period during the processing to food products. A promising strategy to reduce the acrylamide formation would be to decrease the precursors in the raw material. The wide range of variation for asparagine content showed that variety selection might have a large influence on the occurrence of acrylamide in the final product. In addition, the moderately high heritability suggested that successful breeding for lower asparagine content is possible. This conclusion is supported by the observation of no strong negative correlations between asparagine content and a number of other important traits. The genome-wide association mapping resulted in the detection of eight putative QTL, which jointly explained 78.5% of the genetic variance. A putative QTL on chromosome 7B explained with, 18.4%, the highest proportion of the genetic variance for a single marker.

For spelt wheat, we assessed a high number of quality traits but placed a special emphasis on the flavor and odor of bread produced from 30 different varieties. Interestingly, we observed a significant genetic variation for bread flavor and a heritability estimate of moderate magnitude. This suggests that even for bread flavor a successful selection appears possible.

Taken together, for most traits the genome-wide association mapping resulted in the detection of a high number of putative QTL. This indicates a complex genetic architecture, typical for predominantly quantitatively inherited traits. However, few of the putative QTL explained a large proportion of the genetic variance, so that they might have the potential to be used in marker-assisted selection. In order to examine the potential of genomic selection, I performed a five-fold cross validation for the different quality traits. I could confirm previous findings that the integration of QTL information as fixed effects in the genomic prediction model increased the prediction abilities considerably. The average prediction abilities for most traits suggested a high potential for genomic selection in breeding programs.

In conclusion or results form a good basis for further research but more importantly already deliver valuable knowledge that can be used as guideline to advance wheat breeding programs for improved quality.

8 ZUSAMMENFASSUNG

Da Weizen zur Herstellung einer Reihe von unterschiedlichsten Lebensmittel dient, sind Qualitätsmerkmale von großer Wichtigkeit. Brotweizen (*Triticum aestivum* ssp. *aestivum*) wird in Europa in der menschlichen Ernährung vornehmlich zur Herstellung von Gebäck verwendet. Bei Durumweizen (*Triticum turgidum* ssp. *durum*), der fast ausschließlich zur Herstellung von Lebensmitteln, insbesondere Nudelprodukten, verwendet wird, ist die Bedeutung von Qualitätsmerkmalen mindestens genauso groß wie bei Brotweizen. In Mitteleuropa zeichnet sich die Weizenunterart Dinkel (*Triticum aestivum* ssp. *spelta*) im Vergleich zu Brotweizen durch eine besondere Qualität aus. Die Herstellung von Dinkelprodukten beschränkt sich auf einen Nischenmarkt, welcher sich durch ein besonderes Augenmerk auf die Qualität des Endprodukts auszeichnet. Für Weizenzüchter stellt die große Anzahl an Qualitätsmerkmalen, die eine Weizensorte erfüllen sollte, eine große Herausforderung dar. Aus diesem Grund erweist sich Wissen über die genetische Architektur und die Zusammenhänge zwischen den einzelnen Qualitätsmerkmalen als sehr wertvoll für die Weizenzüchtung. Aufgrund der großen Anzahl an Qualitätsmerkmalen bei Weizen, konzentrierte ich mich je nach Weizenart auf eine Auswahl von aktuell wichtigen Qualitätsmerkmalen.

Beim Durumweizen lag mein Interesse bei Merkmalen mit einer großen Relevanz für Durum-Müller und Nudelhersteller. Der Proteingehalt und das Sedimentationsvolumen sind von großer Bedeutung für Nudelhersteller, da diese die Formstabilität, besser bekannt als „al dente“, der gekochten Nudeln beeinflussen. Eine niedrige Fallzahl kann zu braunen statt hellgelben Nudeln führen. Bedingt wird dies durch eine verstärkt ablaufende Maillard-Reaktion während der Nudelherstellung und Trocknung bei niedrigen Kornfallzahlen. Die Glasigkeit der Körner und die Tausendkornmasse gelten als Einflussfaktoren auf die Griesausbeute und sind damit besonders wichtig für Durum-Müller. In der Genom-weiten Assoziationskartierung identifizierte ich eine große Zahl potenzieller QTL. Für das Sedimentationsvolumen stellte sich eine genetische Region auf Chromosom 1B als bedeutend heraus. Eine BLAST (engl. für Basic Local Alignment Search Tool) Recherche gegenüber den Referenz Genomen von Wild Emmer und Brotweizen deutete auf das *Glu-B3* als wahrscheinliches Kandidaten-Gen hin. Bei der Glasigkeit erklärten genetische Regionen auf Chromosom 7A einen größeren Anteil der genetischen Variation. Für einen dieser QTL, der auch einen leichten Einfluss auf den Proteingehalt hatte, deutete die BLAST Recherche auf einen möglichen Zusammenhang mit dem *Pinb-2* Gen hin. Folglich könnte diese genetische Region einen Grund für den positiven Zusammenhang zwischen Glasigkeit und Proteingehalt liefern, den man bisher aus rein phänotypischen Studien kannte. Für die TKM identifizierte ich einen weiteren QTL, der einen großen Anteil der genetischen Variation erklärte, aber keinem bisher bekannten Gen zugeordnet werden konnte. Neben einer guten Leistung in den Qualitätsmerkmalen sollte eine moderne Durumweizensorte auch eine gute agronomische Leistung aufweisen, insbesondere einen hohen Kornertrag. Das stellt eine große Herausforderung für Züchter dar, da der Kornertrag und der Proteingehalt negativ korreliert sind. Zur gleichzeitigen Verbesserung von Kornertrag und Proteingehalt wurden der Proteinertrag oder die Korn Protein Abweichung

(GPD) vorgeschlagen. Wir werteten diese und weitere Selektion-Indices bezüglich ihres Potenzial zur gleichzeitigen züchterischen Verbesserung von Kornertrag und Proteingehalt aus. Unsere Ergebnisse deuteten darauf hin, dass eine erfolgreiche Selektion anhand der Selektions-Indices möglich ist. Jedoch hängt ihre Wirkung stark von der Gewichtung der beiden Ausgangsmerkmale ab. Eine Selektion auf eine hohe GPD würde hauptsächlich zu einem Anstieg im Proteingehalt führen, während eine Selektion auf den Proteinertrag besonders zu einer Verbesserung des Kornertrags führen würde. Nichtsdestotrotz erlaubt eine Kombination der unterschiedlichen Indices eine ausgeglichene Selektion. Im Vergleich zu den Ausgangsmerkmalen unterschieden sich die Selektions-Indices jedoch nicht maßgeblich in der Komplexität der genetischen Architektur.

Beim Brotweizen haben wir ein völlig neues Qualitätsmerkmal betrachtet, den Acrylamid-Vorläufer Asparagin. Acrylamid entsteht in potenziell krebserregenden Mengen, wenn Getreide während der Nahrungsmittelherstellung über einen längeren Zeitraum hohen Temperaturen ausgesetzt ist. Ein vielversprechender Ansatz, um die Acrylamid-Bildung zu reduzieren, ist die Absenkung des Asparagingehalts bereits im Rohmaterial. Die große Variation des Asparagingehalts verdeutlichte, dass bereits die Auswahl geeigneter Sorten zu einer deutlichen Reduzierung von Acrylamid in Endprodukten beitragen könnte. Darüber hinaus lässt eine moderate Heritabilität auf einen möglichen Zuchterfolg für niedrige Asparagingehalte schließen. Auch korrelierte der Asparagingehalt nicht negativ mit anderen wichtigen Merkmalen bei Weizen, was eine erfolgreiche Züchtung erschweren würde. In der Genom-weiten Assoziationskartierung registrierten wir acht potenzielle QTL, die zusammen 78.5% der genetischen Variation erklärten. Ein QTL auf Chromosom 7B erklärte als einzelner Marker mit 18.4% den größten Anteil an der genetischen Variation.

Für Dinkel untersuchte ich eine große Anzahl an Qualitätsmerkmalen, legte aber einen Schwerpunkt auf zwei bisher in der Qualitätsbewertung und -züchtung nicht beachteter Merkmale, nämlich den Geschmack und den Geruch von Broten. Interessanterweise beobachtete ich für den Brotgeschmack von 30 verschiedenen Dinkelsorten eine signifikante genetische Variation und eine Heritabilität mittleren Ausmaßes. Dies könnte sogar für den Brotgeschmack eine erfolgreiche Selektion bzw. Sortenwahl beim Bäcker ermöglichen.

Generell identifizierte ich bei der Genom-weiten Assoziationskartierung für die meisten der untersuchten Merkmale bei Durum- und Brotweizen eine größere Anzahl an QTL. Dies deutet auf eine komplexe genetische Architektur hin, die typisch für vorwiegend quantitativ vererbte Merkmale ist. Jedoch erklärten wenige der QTL einen großen Anteil der genetischen Variation, sodass diese möglicherweise in der Marker-gestützten Selektion eingesetzt werden könnten. Um das Potential von Genomischer Selektion zu untersuchen, führte ich für unterschiedliche Merkmale eine fünffache Kreuzvalidierung durch. Ich konnte vorhergehende Erkenntnisse bestätigen, dass eine Integration von QTL-Informationen als fixe Effekte in das genomische Vorhersagemodell zu einer deutlichen Verbesserung der Vorhersagegenauigkeit beiträgt. Die durchschnittliche Vorhersagegenauigkeit deutete auf ein hohes Potential von Genomischer Selektion in Zuchtprogrammen hin.

Schlussendlich bilden unsere Ergebnisse eine gute Grundlage für weitere Forschung, aber noch viel wichtiger liefern diese bereits jetzt Erkenntnisse, welche als Richtschnur für die Weiterentwicklung von Weizenzuchtprogrammen genutzt werden können.

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DECLARATION

Declaration in lieu of an oath on independent work

according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic "Genetic architecture of quality traits in wheat" is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

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I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

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